OPEN

Assessment of IgM DSAs in Transplant Recipients: Relationship to De Novo IgG DSAs and Risk for Antibody Rejection

Xiaohai Zhang, PhD[1] and Stanley C. Jordan, MD[2]

Background. The presence of anti-HLA donor-specific antibodies (DSAs) is associated with antibody-mediated rejection (AMR) and inferior graft survival. However, recent data suggest that ~50% of AMR episodes are IgG DSA negative and possibly related to non-HLA DSAs. After the initial activation of B cells to alloantigen, IgM is the first immunoglobulin produced. In addition, both IgM and IgG isotopes can activate the classic complement pathway and induce complement-dependent cytotoxicity to allograft targets. Current practices focus on the assessment of IgG DSAs with little concern for the assessment of IgM DSAs. **Methods.** Here, we examined anti-HLA IgM in a cohort of 22 patients who developed de novo IgG DSAs by a modified single-antigen bead-based test. **Results.** We found IgM HLA DSAs developed before IgG DSAs. The median time from the detection of IgM DSAs to the appearance of de novo IgG DSAs was 461 d. Most patients had IgM DSAs against the same HLA-DQ antigens, for which IgG de novo DSAs were also later detected. IgM DSAs were detected in patients with biopsies suspected of AMR. **Conclusions.** The detection of IgM DSAs could be an early indicator of alloimmune responses to allografts before IgG de novo DSAs appear.

(*Transplantation Direct* 2024;10: e1583; doi: 10.1097/TXD.0000000000001583.)

Alloantigens are potent stimulators of B-cell activation and progression to antibody-secreting cells, producing antibodies reactive with allograft targets. During B-cell development, allele polymorphism, gene rearrangement, and somatic mutation of B-cell receptors allow specific and high-affinity recognition of foreign antigens with subsequent generation of high-affinity antibodies.[1] Most naive B cells express membrane-bound IgM. In the initiation of T cell–dependent B-cell activation, IgM B cells go through B-cell maturation and differentiate into effector B cells and secret soluble IgM through mRNA alternative splicing. Some B cells progress through a class switch to become IgG-, IgA-, or IgE-secreting B cells. Importantly, IgM is the first immunoglobulin isotype generated in the humoral immune response.

Development of de novo donor-specific antibodies (DSAs) against HLA antigens is associated with antibody-mediated rejection (AMR) and poor graft survival in solid organ transplants.[2-5] Currently, the recommended testing platforms recommend assessment of anti-HLA IgG antibodies only. The role of other antibody isotypes has not been thoroughly investigated. It has been assumed that low-affinity IgM or IgA anti-HLA antibodies have little or no capacity to injure the allograft. However, a recent study showed that the detection of anti-HLA IgM was a risk factor for acute rejection after lung transplant.[6] In kidney transplantation, the contribution of anti-HLA IgM to graft failure is controversial. Babu et al[7] showed that posttransplant IgM increased the risk of graft failure, whereas Everly et al[8] showed that the detection of IgM DSAs, alone, was not associated with graft loss. Nevertheless, it is likely that the presence of IgM HLA DSAs is a sentinel of early allosensitization and will likely precede de novo IgG HLA DSA development. One important question to answer is the relationship of de novo IgM DSAs with subsequent IgG DSAs and allograft injury.

Here, we tested anti-HLA IgM antibodies in a cohort of patients who developed de novo IgG DSA and determined its ability to predict early allograft injury that could possibly allow earlier intervention, especially in patients who were determined to have no HLA IgG DSAs.

Received 31 August 2023. Revision received 5 December 2023.

Accepted 17 December 2023.

[1] HLA Laboratory, Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA.

[2] Comprehensive Transplant Center, Cedars-Sinai Medical Center, Los Angeles, CA.

Correspondence: Xiaohai Zhang, PhD, HLA and Immunogenetics Lab-SSB 197, 8723 Alden Dr, Los Angeles, CA 90048. (xiaohai.zhang@cshs.org).

X.Z. participated in project planning, experimental design, data analysis, and article preparation. S.C.J. participated in article preparation.

S.C.J. received clinical trial support from CareDx, CSL-Behring, Hansa Biopharma, Amplyx, and Genentech and speaker fees and/or consultation fees from CSL-Behring, Hansa Biophama, Amplyx, and CareDx. The other author declares no conflicts of interest.

Supplemental digital content (SDC) is available for this article. Direct URL citations appear in the printed text, and links to the digital files are provided in the HTML text of this article on the journal's Web site (www.transplantationdirect.com).

ISSN: 2373-8731

DOI: 10.1097/TXD.0000000000001583

MATERIALS AND METHODS

This study was approved by the institutional review board of Cedars-Sinai Medical Center (Pro00047925). The specificity of anti-HLA IgG was determined by the single-antigen bead-based assay (One lambda, West Hills, CA). The mean fluorescence intensity (MFI) of 2500 was used as the cutoff per the clinical testing protocol in our institution. Kidney transplant recipients were monitored for anti-HLA IgG by the single-antigen bead-based test at 1, 3, 6, and 12 mo posttransplantation during the first year and yearly thereafter. Twenty-two kidney transplant patients developed de novo HLA IgG DSA between 2019 and 2021 and had posttransplant samples before the detection of HLA IgG DSA. No patients had preformed HLA IgG DSA before the transplant. Flow and complement-dependent cytotoxicity crossmatches were negative for all 22 patients at the time of transplantation. We examined HLA IgM in these 22 patients. Anti-HLA IgM DSAs were detected by a modified single-antigen beads-based test. In the modified test, PE-conjugated antihuman IgM secondary antibody, instead of PE-conjugated antihuman IgG, was used to detect the amount of human IgM binding on beads. A pooled sera from highly sensitized patients was used as the positive control. A negative control serum from the manufacturer and human AB sera were included as the negative control. The MFI of 1000 and the presence of cross-reactive group antibodies or epitope analysis were used in combination to grade IgM testing. Sera were stored at –80 °C before IgG or IgM HLA antibody testing. To determine the stability of IgM, 6 mo after the initial testing, we retrieved new aliquots of banked sera and retested IgM in 2 samples that showed strong HLA IgM. We did not find significant changes in MFI between 2 test dates (**Figure S1A, SDC,** http://links.lww.com/TXD/A611). Pooled sera as the positive control were included in each IgM test run, and we also did not find MFI changes over time (**Figure S1B, SDC,** http://links.lww.com/TXD/A611).

RESULTS

We found that 17 of 22 patients (77%) who developed de novo IgG DSA were men. The median time of first detection of de novo IgG DSAs was 1545 d posttransplant. Most patients developed de novo IgG DSA within 3000 d posttransplant, with only 4 patients developing de novo IgG DSAs after 3000 d posttransplant (Figure 1).

Next, we determined whether IgM DSAs can be detected before the appearance of de novo IgG DSA. We assessed samples obtained before the appearance of de novo IgG HLA DSAs for IgM HLA DSAs. We found that 9 of 22 patients (41%) demonstrated IgM HLA DSAs in serum samples before IgG de novo DSAs were detected (Figure 2). The median time between the detection of IgM DSAs and the appearance of de novo IgG DSAs was 461 d (interquartile range, 230–1399). Most IgM DSAs were detected within 1.5 y, but IgM DSAs could be detected up to 4 y in patients before IgG de novo DSAs appeared (Figure 3). In terms of specificity, 8 of 9 patients had IgM DSAs against the same HLA-DQ antigens to which IgG de novo DSAs were also later detected (Table 1). MFI values for IgM DSAs were generally low, with the highest MFI at 4774 for anti-HLA-DQ6 IgM, which was detected in patient #9, 239 d before the detection of de novo IgG DSA against HLA-DQ6. Notably, this patient had creatinine increased from baseline 1.0 to 1.9 mg/dL and was diagnosed with acute cellular rejection (Banff 1A) based on the biopsy at the time of detection of IgM DSAs. Of interest, the pathologist felt that AMR was suspected but did not meet Banff criteria and recommended a correlation of DSAs. Here, the IgG DSAs were not detected until 8 mo later.

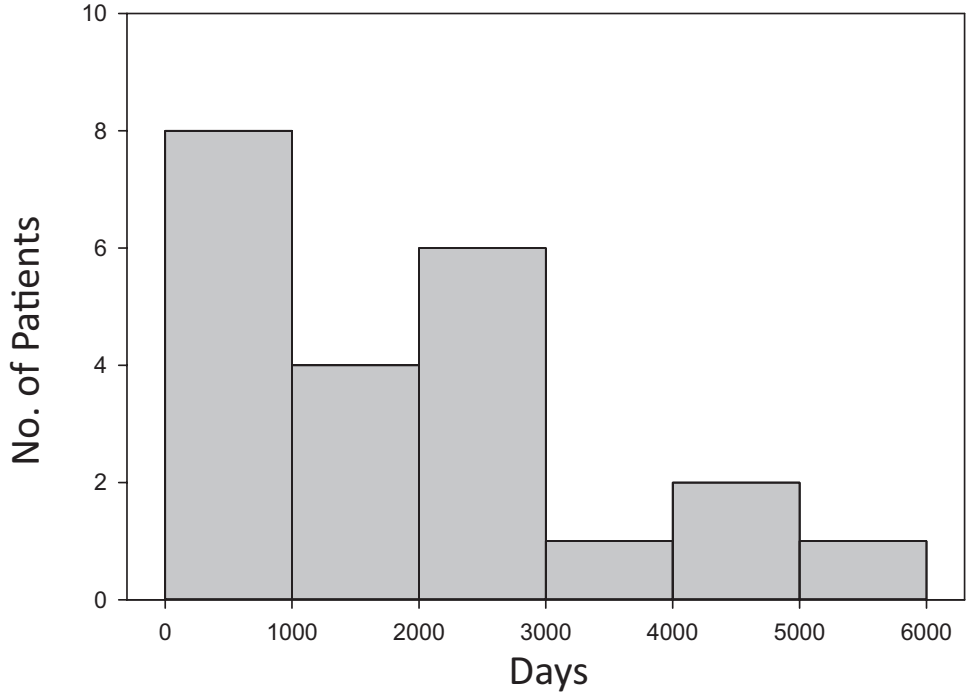

FIGURE 1. Time distribution of de novo IgG DSA detection posttransplant. DSA, donor-specific antibody.

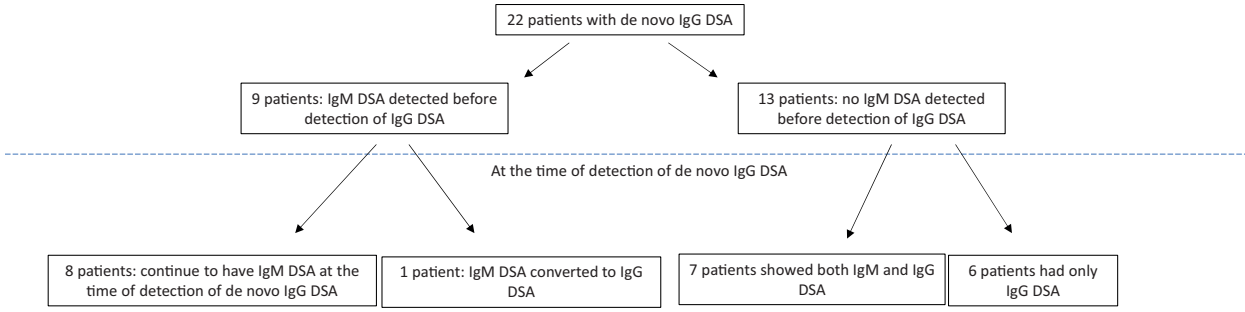

FIGURE 2. IgG and IgM DSA status in the cohort. DSA, donor-specific antibody.

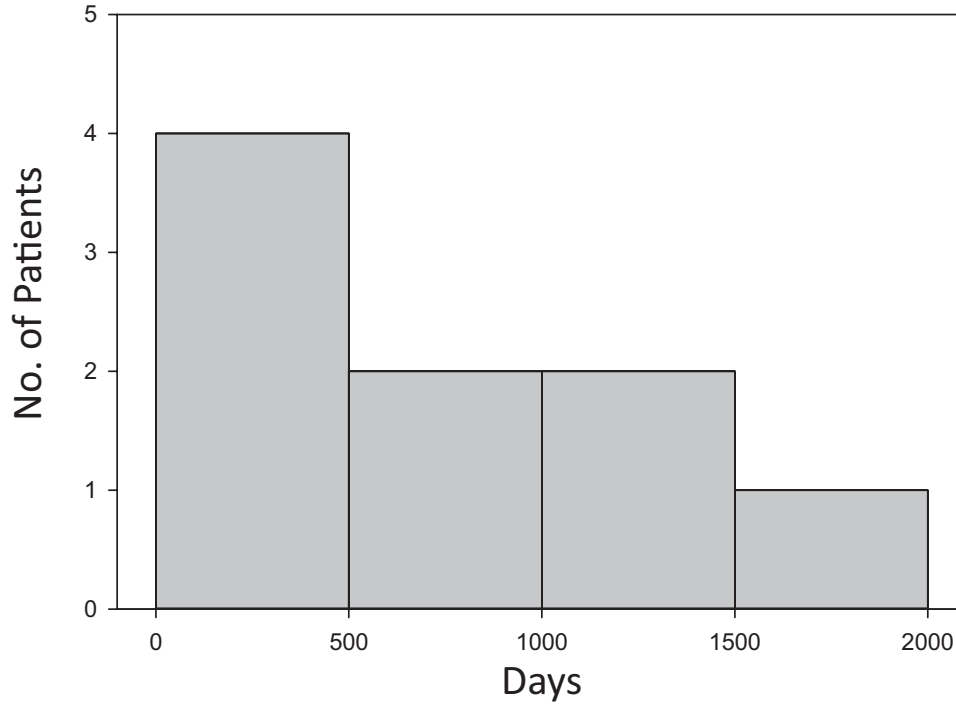

FIGURE 3. Days of IgM DSAs detected before de novo IgG DSA appearance. DSA, donor-specific antibody.

Next, to evaluate the kinetics of donor-specific IgM, we tested anti-HLA IgM in samples when the de novo IgG DSAs were first detected. Eight of 9 patients who had IgM DSAs continued to maintain IgM DSAs when de novo IgG DSAs were first detected (Figure 2). In one patient (patient #6), IgM DSA was not detected at the time of the appearance of de novo IgG DSAs, suggesting that production of the IgM had been switched to the IgG isotype (Table 1). Furthermore, IgM DSAs were detected when de novo IgG DSAs were detected in additional 7 patients who did not have IgM DSAs in the previous samples. Together, IgM DSAs were found in 15 patients when IgG de novo DSAs were detected. In 14 of 15 patients, specificities of IgM DSAs and IgG DSAs shared at least 1 HLA antigen, with most being HLA-DQ antigens. In general, de novo IgG DSAs were broader and stronger than IgM DSAs when both were detected. However, this association was not absolute. For example, patient #22 had a strong anti-HLA-DQ6 IgM DSA, but IgG against the same HLA was weak. In contrast, patient #23 had strong DQA1*05 IgG DSAs but was negative for IgM (Table 1).

We then assessed the relationship of de novo IgM DSA with graft rejection. Of 9 patients who displayed de novo IgM DSAs before the detection of de novo IgG DSAs, only 2 patients (patients #9 and #22) had a biopsy before the IgG DSA detection. AMR was suspected but not firmly diagnosed for these 2 patients because IgG DSAs were not detected at the time of biopsy.

To further confirm IgM DSAs proceeding IgG DSAs, we evaluated IgM antibodies in banked samples from 10 post-transplant patients who did not have IgG DSAs at the time of IgM testing. Eight patients did not display IgM DSAs and 2 patients had IgM DSAs. One IgM DSA-positive patient displayed a weak IgM DSA against HLA-A30. Of interest, we received a new sample for this patient as a routine follow-up after IgM testing, and strong IgG DSAs against HLA-A30 were detected. This new sample was collected 8 mo after the IgM sample. This case supports the notion that IgM DSAs can be detected earlier than IgG DSAs. IgM DSAs against HLA-DQ2 were detected in the second patient, and this patient' serum creatine was 1.84 mg/dL and estimated glomerular filtration rate was 39 mL/min/1.73 m^2. No recent samples were available for the second patient to determine whether the IgM HLA-DQ2 DSAs would switch to IgG DSAs.

TABLE 1.

MFI of donor-specific IgG and IgM in each patient

Patient ID	Gender	Days of de novo IgG DSA posttransplant	IgG DSA (MFI)	IgM DSA (MFI)	Days of IgM DSA tested before IgG DSA Detected	IgM DSA (MFI) detected before de novo IgG DSA
1	F	2820	DP19 (11 366); A2 (1868)	DPB1*04:02 (2971); A2 (1096)	1130	DPB1*04:02 (1270)
3	M	1476	DQA1*05 (23 272)	DQA1*05 (2559)	769	–
4	M	286	DQB1*05:01 (3771)	DQB1*05:01 (1519)	125	DQB1*05:01 (1532)
5	M	2940	DQ6 (5940)	DQ6 (1715); B37 (3246); B49 (1377)	1489	DQ6 (1731)
6	F	3390	DQ5 (5636)	–	1507	HLA-DQ5 (1643)
8	M	4512	DQA1*03 (19 138); DQ4 (9520), DQ9 (16 932)	DQA*03 (1657)	683	–
9	M	1162	DR13 (23 816); DQ7 (22 563); DQA1*05 (23 845); DR11 (6558); DQ6 (3853)	DQA1*05 (9266); DR13 (3317); DQ6 (5155)	239	DQ6 (4774)
10	M	559	DQ5 (13 055); DR53 (5025); DQ8 (6828); A2 (2530)	–	436	–
12	M	358	DQ7 (21 275); DQA1*04 (21 663)	DQA1*04 (4145)	253	–
13	F	356	DQ8 (19 505); DQA1*03 (6641); DR53 (4781)	B49 (4535)	75	–
14	F	2803	DR7 (3273)	–	266	–
15	M	4997	DQ7 (23 523); DR16 (5850)	DQ7 (3385)	384	–
16	M	355	A3 (6711)	–	250	–
17	M	2042	DQ7 (11 318)	DQ7 (2836)	944	DQ7 (4028)
18	M	5200	DQ7 (5217)	–	889	–
19	M	1289	DQA1*04 (17 711); DQ4 (8946)	DQ4 (5771)	552	DQ4 (1331)
20	M	1615	A29 (2595); DQ4 (1477)	–	86	–
21	M	2998	DQ7 (22 977)	DQ7 (15 084)	369	DQ7 (1108)
22	M	575	DQ6 (4262)	DQ6 (1374)	227	DQ6 (1846)
23	M	2234	DQA1*05 (18 560)	–	394	–
24	F	491	DQ2 (18 154)	DQ2 (2421)	119	–
25	M	357	DQ2 (23 841); DQ8 (20 129); DQA1*03 (19 290); DR53 (24 058); DR4 (6415); DP3 (6953)	DQ2 (2619), DR53 (4419); DP3 (1442)	140	–

DSA, donor-specific antibody; MFI, mean fluorescence intensity.

DISCUSSION

Both preformed and de novo DSAs can cause graft injury and shorten graft survival. In the current practice, only anti-HLA IgG antibodies are evaluated for transplant patients. IgM is the first antibody isotype produced by B cells in immune responses. In this study, we showed that anti-HLA IgM detection in patients who ultimately developed de novo anti-HLA IgG could be demonstrated at a median of 461 d before detection of de novo HLA IgG. We found that antidonor HLA IgM DSAs were detected before the appearance of de novo donor-specific IgG in 41% of patients.

Anti-HLA IgM was tested by a modified single-antigen bead-based test in which the secondary PE-conjugated anti-IgG antibodies were replaced with anti-IgM antibodies. We observed the patterns of high levels of nonspecific binding more frequently in the IgM test than in the IgG test. Several IgM test results showed IgM against the patient self-HLAs or against all HLA-DR (**Figure S2, SDC,** http://links.lww.com/TXD/A611). Because of this, analysis of anti-HLA IgM cannot solely rely on a cutoff, and we also had to consider reaction patterns, such as cross-reactivity groups or epitopes, to assign specificities (**Figure S3, SDC,** http://links.lww.com/TXD/A611). Most antidonor HLA IgM identified in our study were against HLA-DQ. An example of HLA-DQ IgM was shown in **Figure S4** (**SDC,** http://links.lww.com/TXD/A611). This finding is consistent with the previous results that most de novo IgG antibodies are against HLA-DQ, and the presence of DQ DSAs is associated with a significant risk of AMR.[2] This suggests that anti-IgM DSAs identified in our cohort could be pathogenic or, at least, a harbinger of evolving humoral pathology.

The presence of IgM with MFI >2000 within 30 d posttransplant has been associated with graft failure in kidney transplantation.[7] The recipients in this study were transplanted across positive crossmatches and are at higher risk of rejection compared with our cohort. In our current study, patients did not have DSAs pretransplant. Nevertheless, both studies suggest that IgM antibodies can play a role in the activation of humoral immunity against allograft. Everly et al[8] showed that the median time of IgM DSA appearance was 2 mo posttransplant, and the median time of IgG DSAs was 10 mo,which is consistent with our observation that IgM can be detected before IgG DSAs. However, they showed that IgM DSAs with MFI >1000 alone did not increase the risk of allograft loss whereas the development of both IgM and IgG DSAs had a higher risk of graft failure in kidney transplantation.[8] This difference may be caused by how IgM DSA results were analyzed. Due to the nature of high nonspecific binding of the IgM test, using the lower cutoff alone may lead to false-positive IgM DSA reaction, which weakens the association of IgM DSAs with the graft outcome. It is well established that the presence of IgG DSAs is associated with allograft rejection. Conversion of IgM to IgG needs T helper cells. Everly et al[9] showed that blockade of T-cell costimulatory pathway by belatacept prevents convention from IgM DSAs to IgG DSAs compared with patients treated with cyclosporine A. These studies indicate that the development of IgM DSAs at an early time might lead to the graft injury, but this can be prevented by blocking T-cell costimulatory signals.

IgM DSAs were not detected in 10 patients in the current study, although they all developed de novo IgG DSAs. The posttransplant samples were collected as routine clinical monitoring. IgM may switch to IgG in a short period, and we missed this small window. However, IgM DSAs can last >2 y in some patients. Donor-specific HLAs may continue to stimulate the recipient immune system to generate new B-cell clones in these patients or IgM DSAs are secreted by long-lived plasma cells.

The major limitation of this study is the small sample size. Only 25 patients developed de novo DSAs in our center over a 3-y period (2 patients did not have pre–de novo DSA samples collected and were not included in the study). We only tested IgM at 2 time points on these 22 patients. IgM testing on samples collected from patients without de novo DSAs in the same period would provide a comprehensive picture of the impact of IgM DSAs on the graft outcome. Studies on larger cohorts are warranted in the future.

Given the increasing number of patients who present with anti-HLA IgG DSA-negative AMR, analysis of de novo IgM DSAs may provide an early signal in assessing the risk for AMR, which would enable early detection and intervention. To validate these findings, a further study is needed to perform an analysis of larger cohorts of patients with defined biopsy findings and negative IgG DSAs to determine whether added benefit could be obtained by early detection of de novo IgM HLA antibodies.

REFERENCES

1. Cyster JG, Allen CDC. B cell responses: cell interaction dynamics and decisions. *Cell*. 2019;177:524–540.
2. Willicombe M, Brookes P, Sergeant R, et al. De novo DQ donor-specific antibodies are associated with a significant risk of antibody-mediated rejection and transplant glomerulopathy. *Transplantation*. 2012;94:172–177.
3. Ginevri F, Nocera A, Comoli P, et al. Posttransplant de novo donor-specific HLA antibodies identify pediatric kidney recipients at risk for late antibody-mediated rejection. *Am J Transplant*. 2012;12:3355–3362.
4. Zhang X, Kransdorf E, Levine R, et al. HLA-DQ mismatches stimulate de novo donor specific antibodies in heart transplant recipients. *Hum Immunol*. 2020;81:330–336.
5. Le Pavec J, Suberbielle C, Lamrani L, et al. De-novo donor-specific anti-HLA antibodies 30 days after lung transplantation are associated with a worse outcome. *J Heart Lung Transplant*. 2016;35:1067–1077.
6. Miyahara K, Miyoshi K, Kurosaki T, et al. Circulating anti-human leukocyte antigen IgM antibodies as a potential early predictor of allograft rejection and a negative clinical outcome after lung transplantation. *Surg Today*. 2022;52:52–60.
7. Babu A, Andreou A, Briggs D, et al. Clinical relevance of donor-specific IgM antibodies in HLA incompatible renal transplantation: a retrospective single-center study. *Clin Transpl*. 2016;32:173–179.
8. Everly MJ, Rebellato LM, Haisch CE, et al. Impact of IgM and IgG3 anti-HLA alloantibodies in primary renal allograft recipients. *Transplantation*. 2014;97:494–501.
9. Everly MJ, Roberts M, Townsend R, et al. Comparison of de novo IgM and IgG anti-HLA DSAs between belatacept- and calcineurin-treated patients: an analysis of the BENEFIT and BENEFIT-EXT trial cohorts. *Am J Transplant*. 2018;18:2305–2313.